PVA- BENTONITE COMPOSITE FOR WOUND DRESSING

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CERTIFICATE

This is to certify that the thesis entitled **"PVA bentonite composite for wound dressing"** is a record of the bonafide work done by KIRAN YELLAPA VAJANTHRI (212BM2017) which is submitted for partial fulfilment of the requirements for the degree of Master of Technology (MTech) in Biotechnology at National Institute of Technology, Rourkela. To the best of my knowledge, the matter embodied in the thesis has not been submitted to any other University/Institute for the award of any Degree or Diploma.

Place: NIT Rourkela
Date: 25 May, 2014

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ABSTRACT

Wound healing is a complex process involving various systems of the body like circulatory system, immune system and the actions of epithelial cells. Wound dressings are used to accelerate the process of wound healing. An ideal wound dressing must have the characteristics of providing moist environment, an ability to absorb the wound fluids, protection from secondary microbial infections and most importantly it must be biocompatible. In the current study two types of wound dressing materials were synthesized one with the compositions of PVA and organically modified bentonite (OBENT) and other with PVA, OBENT and honey. Bentonite clay provided the suitable mechanical, chemical and physical properties for the dressing; honey provided the antimicrobial properties along with skin beneficiary properties that it naturally possess. Further the dressings prepared were comparatively analyzed by various characterization techniques like scanning electron microscopy (SEM), Fourier transform infrared (FTIR) spectroscopy and X-ray powder diffraction (XRD) techniques. The wound dressing samples were subjected to swelling, water vapour transfer rate and antimicrobial studies. From the above studies, it can be concluded that PVA-OBENT composites without honey could be used for high fluid and exudate releasing wounds whereas PVA-OBENT composites with honey are suitable for dry wounds.

Keywords: PVA, BENTONITE, HONEY, COMPOSITE, WOUND DRESSING

CHAPTER 1 INTRODUCTION

Wound is a condition of the skin where in its normal structural integrity is damaged due to mechanical, chemical, physical interactions of body with the external environment. Wound healing is body`s response to maintain its status quo. It involves various organisational levels of the body starting from extracellular matrix (ECM) of the cells to various systems of the body like integumentary system (skin, sweat glands, hair, nails and sebaceous glands), circulatory system (heart and blood vessels) and immune system etc. The process basically involves four stages homeostasis, inflammation, proliferation and remodelling. Homeostasis is the process of wound healing involving the platelets which along with the clotting cascade proteins like thrombin and fibrin seal the damaged parts by forming clots. This essentially stops the bleeding. Homeostasis is followed by inflammation which is characterised by classical symptoms of redness, swelling, heat and pain in the wound area. Redness and heat in the wound area is due to the expansion of vessels or vasodilation. Swelling occurs due to accumulation of wound exudates within the barrier formed by the platelet and fibrin mesh meant for prevention from further infections. The wound exudate consists of the various cells like macrophages, monocytes and granulocytes which remove tissue debris and foreign organisms. The blood clotting factors release kinins which cause pain. Proliferative phase involves the migration of epithelial and fibroblast cells to the wound area. Epithelial cells move from the margins of the wound and form a thickened epidermal cap subsequently proliferation of the cells takes place. Granulation tissue is formed by in growth of capillaries, lymphatic vessels and collagen strengthening the skin. Remodelling phase involves the strengthening of tissue and scar formation [1]. Wound dressings are medical devices that are used to enhance the rate at which wound healing process occurs. Wound dressings must ideally have characteristics like maintenance of moisture, permeability of gases, protection against secondary infection, thermal insulation, non-antigenic, elastic, biodegradable and biocompatible. Wound dressings could be classified into various types like hydrocolloid dressings, alginate dressings, hydrofiber dressings, foam dressings, antimicrobial dressings and hydrogel dressings. Hydrocolloid dressings have carboxy methylcellulose, pectin and gelatin as its basic material which form gel on contact with wound fluids and usually used for low or moderately exudative wounds. Alginate dressings derived from seaweeds have been found to be haemostatic due to Ca^{++} ions so can be used in bleeding wounds.

Hydrofiber dressings are formed of textile fibers with sodium alginate they from gel on exposure to wound exudate, they have properties of both hydrocolloid and alginate dressings and have addressed the weakness of cohesive gelling and aggressive adhesion [2]. Foam dressing material consists of polyurethane and is used in cases of high exudative wounds. Antimicrobial dressings are basically incorporated with agents like silver, iodine and honey in order to counter the increasing antibiotic resistance in various wound microbes [3].

1.1 Hydrogels

Hydrogel consists of two words hydro meaning water, gel meaning a state which is in between that of solid and a liquid. Hydrogels are cross linked networks of hydrophilic polymers that have the capacity to absorb water and attain equilibrium. The network formation is due to the physical and chemical linkages within the components. Physical linkages occur due to presence of crystalline regions or the secondary interactions like electrostatic forces, hydrophobic forces etc. whereas chemical interactions are represented by covalent bonds. The various hydrophilic polymers used include polyvinyl alcohol, polyvinyl pyrrolidone, poly ethylene glycol, poly acrylic acid, polyacrylamide etc. Hydrogels have been synthesized by mainly three methods which include the conventional physical crosslinking, chemical crosslinking and radiation based method of cross linkage. Physical or conventional method involves usage of freezing and thawing cycles which gives crystalline nature to the product, the chemical method involves the usage of various crosslinking agents like glutaraldehyde, acetaldehyde, formaldehyde etc., for network formation. Radiation based method involves high energy radiation exposure of polymers creating free radicals which lead to network formation. The network formation between polymers gives the hydrogel its 3D structural arrangement. The degree of cross linkage in hydrogels decides the swelling ratio, shear modulus and diffusion coefficient of embedded molecules. Biocompatibility properties depend upon the initial chemicals being used for product processing [4-6].

The ability to maintain a 3D structural arrangement similar to biological tissues led to first introduction of hydrogels as a biomaterial. Moist wound healing studies by George Winter and the various studies where it was seen that the hydrogels had the properties of water retention, hydrophilic nature, biological inertness, metabolite transportation ability and

painless removal pointed towards its usage as a wound dressing [6]. Hydrogels have also been found to be fulfilling various ideal wound dressing characteristics like avoidance of open wound infections, maintenance of moisture, absorption and retention of exudates, thermal insulation, incorporation of suitable drug, gas permeability, painless removal and better wound monitor [6-8]. Hydrogels have been found to be associated with weak elastic and mechanical properties. So, in order to solve the problem they have been mostly found to be used as composites [8-10].

1.2 Composites

Composites are materials formed by combination of matrix and reinforcements which not only retain their inherent properties but, also show new characteristics in the end product making it better than its constituting individual components. Matrix forms continuous phase in which the reinforcements are dispersed and surrounded by matrix. A composite also consists of another phase called the interphase which is a new phase formed due to combination of the matrix and reinforcements. The performance of the composite thus formed is determined by the degree and extent of chemical and physical forces acting between the components stabilizing the multiphase system. Composites can be classified on the basis of material of the matrix, dispersed phase form and type of reinforcing fibres. Based on matrix material of the composites they can be classified as metal composites, polymeric composites, and non-metallic materials. Dispersed phase form based classification includes continuously fibre reinforced composites, fibrous fabric reinforced composites, whisker reinforced composites, sheet reinforced composites, particle reinforced composite and nanoparticles reinforced composite. Composites with respect to reinforcing fibre type can be subdivided into organic fibre, glass fibre, boron fibre, hybrid fibre and carbon fibre [11].

1.3 Polymeric matrix composite hydrogels:

Polymeric matrix composites belong to the class of matrix based classification of composites. Composite hydrogels are materials which consist of two or more components viz; the polymeric matrix which forms the major part of the composite and reinforcing filler materials which are usually inorganic nanoparticles. These inorganic nanoparticles have at least one dimension in nano size. The formed polymer nanocomposites have better mechanical (tensile strength, compressive strength and hardness), water vapour barrier,

chemical resistance and thermal stability properties. The type of reinforcements include: nanoclays, carbon nanofibers, carbon nano tubes (CNTS), nanosilica, nanoaluminium oxide (Al_2O_3) and nanotitanium oxide (TiO_2). In the current work, composite hydrogels intended to be used for wound dressing purposes have been synthesized by using a polymeric matrix of poly vinyl alcohol (PVA) and inorganic nanoparticles in the form of bentonite clay.

1.4 Poly vinyl alcohol (PVA)

Poly vinyl alcohol (PVA) is a synthetic, hydrophilic and thermosetting polymer which is synthesized by free radical polymerisation of vinyl acetate to poly vinyl acetate followed by hydrolysis. The degree of hydrolysis does not usually complete so; the commercially available is referred to a certain degree of hydrolysis (generally above 98.5%). The degree of hydrolysis determines the various properties like chemical reactivity, solubility and crystallinity. The higher the degree of hydrolysis, higher will be the acetate groups which results in lower solubility level and increases difficulty in crystallization of the polymer. However PVA applicability has increased due to the polymers particular properties such as water solubility, biodegradability, biocompatibility, nontoxic nature, non-carcinogenic, non expensive nature, and possesses a high degree of flexibility which allows for tailor made products. It has been used in various biomedical applications like medical sutures, artificial meniscus, drug delivery devices, contact lenses, implants etc. PVA has also been used in various industries like foods, adhesives, paints, resins and cosmetic industries [12-13]. The polymer has the ability to form hydrogels by chemical, physical and radiation based methods. The physical crosslinking method has been found to be very useful as it does not produce any residual toxicity during the course of application which is relevant in case of chemical cross linking methods. The physical method for hydrogel synthesis involves repeated freeze and thaw cycles of PVA solutions. During the freeze cycle ice formation takes place in the solution followed by polymeric network formation and crystallisation until an overall crystallite formation completes throughout the solution. During thaw process ice melts lead to pores and finally hydrogel formation [14]. The crystalline nature of the hydrogel increases as the number of freeze thaw cycles increases. It also depends upon the temperatures and durations of time for freeze and thaw cycles. Other factors that affect hydrogel formation include the concentration of PVA and the molecular weight of the polymer. It has been reported that

the physically cross linked hydrogels possess better swelling, stiffness, elasticity and strength than chemical based hydrogels [4, 15]. One of the major setbacks with respect to physically crosslinked hydrogels despite their excellent biological tissue like nature is their insufficiency with respect to their mechanical properties limiting their wound dressing usage [8-9]. In order to improve PVA hydrogels, inorganic particles like nanoclays have been used as reinforcements.

1.5 Bentonite clay

Bentonite clay is one among the various nanoparticles reinforcements used for polymer composites. Bentonite is naturally occurring clay formed due to *in-situ* alteration of volcanic ash and rocks. Bentonite clay has montmorillonite as its main component. Along with montmorillonite, it contains other clays and chemicals like illite, kaolinite, quartz and carbonates. Bentonite clay belongs to a group of 2:1 phyllosilicates and smectites familiy. Their crystal structure consists of stacked layers made of two silica tetrahedrons sandwiching an edge-shared octahedral sheet of alumina. The tetrahedral sheet of silica layer consists of SiO_4 groups linked to form hexagonal network of Si_4O_{10}. The tetrahedral alumina layer consists of two sheets of closely packed oxygens or hydroxyls between which octahedrally coordinated aluminium atoms are equidistantly present from six hydroxyls or oxygens. All these three layers form a layered sheet system. The thickness of each layer is approximately 1 nm and its length may vary between 300Å to several microns. The adjacent layers are separated by gap called as the interlayer or gallery. Due to its composition the aluminium atoms get replaced by magnesium causes difference in valences of Al and Mg, which in turn creates negative charges distributed within the plane of the platelets that are balanced by positive counterions, typically sodium ions, located between the platelet galleries. Formation of polymer composites is only possible with hydrophilic polymers. In order to make the inorganic clay compatible with organic polymers, the clays are modified organically by exchanging the interlayer or gallery cations with organic cationic surfactants like quaternary ammonium salts, alkyl imidazoles and cationic phosphonic compounds. Cationic surfactants have long aliphatic tails and cationic heads which interact with surface of the negatively charged silicates, thus leading to larger interlayer spacing. The organic modification lowers the surface energy of inorganic clay particles, improving their wettability with the polymer matrix [16-18]. Polymer composites formed may be classified into intercalated, exfoliated and

phase separated states, on the basis of degree of dispersion and interaction of the clay within the polymer matrix. Phase separated composites occur when there is absolutely no interaction between the clay and polymer. A good interaction between polymer and clay is to exist when the clay galleries have expanded to space occupation by polymers. Best condition for polymer composites exists when the clay plates have completely dispersed and separated from each other.

1.6 Honey

The advent of penicillin antibiotic by Alexander Fleming was one of the great discoveries which lead to development of health care. Since then many antibiotics have been introduced and used as medicines. One of the major concerns today is number of the antibiotic resistant strains of microbes that are coming into existence and the problem adds up with very less number of new antibiotics being invented. Honey has been used in many ancient civilizations for various medicinal purposes. In lieu of the antibiotic resistance situation honey has been found to be having great effect on antibiotic resistant microorganisms. So, usage of honey as antimicrobial agent becomes relevant. The antibacterial properties of honey have been due to the presence of low pH, high sugar concentration, hydrogen peroxide, methylglyoxal and the antimicrobial peptide bee defensin-1. Thus honey can be used in wound dressings to enhance the antibacterial properties [19].

In the current work, PVA Bentonite composites with and without honey was synthesized and characterized using scanning electron microscopy (SEM), X-ray diffraction (XRD) and fourier transform infra red spectroscopy (FTIR). The composites were also evaluated for wound dressing behaviour by conducting swelling, water vapour transmission rate and antibacterial studies.

CHAPTER 2 LITERATURE REVIEW

2.1 Wound dressings

Wound healing is a complex process involving various systems of our body. Wound dressing have been used to enhance the rate of wound healing. Provision of moist environment by a wound dressing has been shown to enhance the epithelisation and in turn increasing wound healing rate. The importance of moist healing was proved by G. Winter`s work in the year 1962. Wound dressings need to provide certain ideal wound dressing characteristics that include no permeability to water and bacteria, freedom from particulate matter, thermal insulation, absorption and retention of exudate, Prevention of trauma on removal, removal of toxic substances, prevention of dehydration, allow for gaseous exchange and painless removal of the dressing [20]. Hydrogels have been found to be satisfying these ideal wound healing characteristics. So, Hydrogels are used as wound dressing materials. Hydrogels provide moisture to dry wounds as well as absorb excess exudate, depending on moisture levels at the wound hydrogels. Depending on the type of hydrogel, they contain varying percentages of water, but do not altogether dissolve in water. Despite their high water content, hydrogels are capable of additionally binding great volumes of liquid because of the presence of hydrophilic residues. Hydrogels swell extensively without changing their gelatinous structure and are available for use as amorphous gels and in various types of application systems. Most hydrogels have a high water content of approximately 70% and it is this factor that successfully promotes rehydration.

Hydrogels also have the ability to absorb some fluids from low exuding wounds. However, if used in sloughy and highly exuding wounds, hydrogels can begin to donate fluid to the wound [21]. Hydrogels have been synthesised by basically three methods physical cross linking, chemical crosslinking and radiation based methods. The physical crosslinking method involves subjection of the polymeric solution to repeated freeze – thaw cycles. The time duration of freezing and thawing, temperature of freezing and thawing determine the polymeric network formation in the hydrogel. Chemical crosslinking the polymeric solutions are added with cross linking agents like acetaldehydes, formaldehyde, glutaraldehyde, boric acid and borax. Radiation based hydrogels are synthesized by using electron beam radiation, gamma radiation which

provide the crosslinking as well as the sterilisation to the hydrogels. Physical crosslinked PVA hydrogels were synthesized and characterised by x-ray diffraction, mechanical testing, swelling tests, surface energy measurements and cell culture experiments. The PVA hydrogels formed by the method have been proposed to be having desired features like biocompatibility, surface of cell attachment, strength and elasticity required for tissue engineering applications [14]. Chemically cross linked PVA hydrogels have been synthesized by using glutaraldehyde in acidic HCl medium. These PVA hydrogels were prepared using different degree of hydrolysis in water, solvents and solvent mixtures. These gels were characterised with respect to FTIR and XRD and it was found that glutaraldehyde usage causes toxicity and irregularity in the obtained matrix [22]. Radiation based sterculia gum- PVA–PVP hydrogels have been prepared through radiation crosslinking; the hydrogels were used antimicrobial agent carrier to the wounds. The radiation crosslinked hydrogels were characterized with respect to SEM, FTIR, TGA (thermogravimetric analysis) and swelling studies. It was found that network formation due to radiation influenced the structure of the hydrogels which in turn enhanced the antibacterial carrier abilities and the hydrogels thus formed were concluded to have potential as wound dressings [23].

2.2 PVA hydrogels

PVA hydrogels have been used in various biomedical applications like contact lenses, artificial meniscus, artificial pancreas, wound dressings, hydrophilic catheters, cartilage replacements etc thus providing an idea that they have been successful in providing natural tissue like environment due to the reasons of biocompatibility chemical inertness and water absorption and low protein adsorption characteristics. PVA has additional properties of processing into transparent, flexible, economical hydrogels [12, 24]. First radiation based PVA hydrogels were proposed as wound dressings by rosiak *et.al* in 1991 [6]. PVA has been found to be suitable for all the three methods of synthesis of hydrogels i.e physical crosslinking, chemical crosslinking and radiation based cross linking [8, 14, 22-23]. Physical crosslinking method of hydrogels has been found to be most suitable due to the absence of toxic crosslinkers (e.g. glutaraldehyde). PVA hydrogels with excellent properties have been synthesized by freeze thaw method with 15 (w/v) % PVA concentrations, freezing at a temperature of -20^0C and thawing at 23^0C for 24 hours each.

PVA hydrogels have been associated with problems with respect to their elasticity and mechanical strength which hinders their usage as wound dressing [8-9]. In order to improve these properties hydrogels are being synthesized in the form of composites having PVA with polysaccharides and inorganic particles. One of the inorganic particles used are the clays like bentonite which have to be organically modified to have better compatibility with PVA like hydrophilic polymers otherwise this may lead to phase separated mechanically weak hydrogel composites. Bentonite clay have layered structure of two tetrahedral Silicon linked to oxygen or hydroxyl sheets that sandwich a octahedral aluminium which is again linked with hydroxyl and oxygen groups. Distance between two layers of clay is called as gallery or inter layer space. When clays are organically modified and interacted with polymers, the polymeric matrix intercalate within the galleries leading to polymer composite formation. Such PVA clay composites have been reported [18, 25].

One of the recent reports includes on the formation of composites in which different montmorillonite clays obtained from southern clay products were casted along with poly vinyl alcohol to form composite films. The films formed were characterised by, X-ray diffraction, differential scanning calorimetry, thermogravimetric analysis, transmission electron microscopy, water absorption, contact angle, and mechanical properties. It was found that composites with improved mechanical, water absorption, surface energy could be obtained [26]. PVA based composite hydrogels showing potential with respect to their wound dressing behaviour have been reported [8]. PVA bentonite composite hydrogels have been synthesized and characterised mechanically by tensile testing, the wound dressing capabilities have been evaluated by water vapour transmission rate and antibacterial assay. The results show hydrogel has potential for wound dressing as it had WVTR values in between those for normal skin and damaged skin, appropriate amount of antibacterial properties against *Listeria* and *E.coli.* PVA and organically modified montmorillonite hydrogels have been prepared by using freeze thaw method [8]. The modified montmorillonite has been varied from 0, 2, 5, 7 and 10 % (w/w) with respect to weight of PVA taken. The various hydrogels obtained were characterised by using methods like TEM, XRD, tensile testing, hardness testing, swelling, water vapour transmission rate and microbial penetration properties. The results showed that the obtained hydrogels were concluded to be having excellent potential for wound dressing [8]. Wound dressing has important role in prevention of secondary infections in wounds.

For this purpose various antibiotics could be incorporated within the hydrogels. But, the increasing number of antibiotic resistant microbes is a growing concern. In order to tackle the situation honey could be incorporated within the hydrogels. Honey at a concentration of 40% has been proven to have antimicrobial properties against various antibiotic resistant microbes like *B. subtilis, S. aureus.* and *P. aeruginosa* like bacteria. The various components which have been responsible for the antibacterial nature include high sugar contents, low pH, hydrogen peroxide, bee defensin protein and methylglyoxal [19, 27]. In the present research work, PVA bentonite hydrogels with and without honey have been prepared by conventional freeze thawing method. The hydrogels were characterized materially by SEM, XRD and FTIR. The wound dressing behaviour was tested by performing swelling, WVTR and antibacterial assays.

CHAPTER 3 MATERIALS AND METHODS

3.1 Materials

Poly vinyl alcohol (PVA), bentonite, mannitol, sodium chloride (NaCl), and cetyl-triammonium bromide (CTAB) were purchased from Himedia. The honey being used was purchased from local consumer store. Nutrient broth and nutrient agar used in this study was purchased from SRL chemicals.

3.2 Methods

3.2.1 Organic modification of bentonite

1% (w/v) suspension of bentonite in 1 litre of distilled water was prepared. The obtained bentonite suspension was stirred and heated at 80^0C for 24 hours. Another solution of 5% (w/v) CTAB solution was prepared in 100 ml of distilled water. The CTAB solution is added drop wise, stirred until 10 hours in order to complete the cationic exchange. The clay thus obtained is washed and filtered thoroughly to remove the unexchanged clay. Thus obtained clay is dried and stored in zip-lock to prevent from moisture. The clay will further be referred to as organically modified bentonite (OBENT).

3.2.2 Preparation of PVA bentonite composite hydrogels

PVA 15% (w/v) in water was added with different concentrations of organically modified Bentonite (OBENT) viz. 5, 10, 15 and 20 percentages by weight (w/w) with respect to PVA and stirred at a temperature of 90^oC for 4 hours. The obtained polymeric composite is poured into glass moulds and kept for freezing in refrigerator at -20^oC for 24 hours. After 24 hours the moulds were kept for thawing in water at room temperature for 2 hours. This completes one cycle of freeze thawing cycle. This cycle was repeated for two more times to obtain PVA-OBENT composite hydrogel.

3.2.3 Preparation of PVA bentonite composite hydrogels with honey

PVA 15% (w/v) in water was added with different concentrations of organically modified bentonite (OBENT) viz. 5, 10, 15 and 20 percentages (w/w) with respect to PVA and stirred at a temperature of 90^oC for 3 hours. Next the mixtures were added with 80% (w/v) honey and stirred at a temperature of 90^oC for 1 hour. Next the liquid is poured into glass moulds and kept for freezing in refrigerator at -20^oC for 24 hours. After 24 hours the

moulds are kept for thawing in water at room temperature for 2 hours. This completes one cycle of freeze thawing cycle. This cycle was repeated for two more times to obtain PVA bentonite composite hydrogel with honey.

Table 1 PVA Bentonite composites and their sample codes

SL NO	SAMPLE COMPOSITION	SAMPLE CODE
1	PVA	PVA
2	PVA+5%OBENT	PB05
3	PVA+10%OBENT	PB10
4	PVA+15%OBENT	PB15
5	PVA+20%OBENT	PB20
6	PVA+Honey	PH
7	PVA+Honey+5%OBENT	PHB5
8	PVA+Honey+10%OBENT	PHB10
9	PVA+Honey+15%OBENT	PHB15
10	PVA+Honey+20%OBENT	PHB20

3.2.4 X-ray diffraction studies

The X–ray diffraction study was carried out on Rigaku Ultima IV diffractometer. Bentonite (BENT), organically modified bentonite (OBENT) and the various PVA-Bentonite hydrogel samples with and without honey were scanned from 1 to 30^{o} at a scanning rate of 5^{o}/min.

3.2.5 Scanning electron microscopy (SEM)

The surface morphology of the various samples was studied after serial dehydration of the PVA bentonite composite samples. The samples were cut by 0.5 X 0.5 cm in dimensions and serially dehydrated. The samples were dipped in 3 ml of 70% (v/v) ethanol solution for 20 minutes. The samples were removed from 70% (v/v) ethanol solution and transferred to 3 ml of 80% (v/v) ethanol solution for 20 minutes; similarly the samples were subjected to 90% and 95% ethanol solutions respectively for 20 minutes each. Finally the samples were dipped in 3 ml of 100% ethanol and removed after 1 minute. The serially dehydrated samples were observed in SEM (Nova NANOSEM 450) to study the surface morphology.

3.2.6 Fourier transform infrared spectroscopy (FTIR)

FTIR spectra of PVA bentonite composite hydrogel samples were measured using Perkin Elmer IR spectrophotometer. The samples were dried and powdered. Potassium bromide (KBr) pellets for each of the samples were prepared. The measurements were taken between the ranges of 400 to 4000 cm^{-1}.

3.2.7 Swelling Studies

For swelling studies samples were cut and initial weight was determined (Wi). The samples were then further kept in distilled water at 37^{o}C to study swelling behaviour. The samples were taken out from distilled water at regular intervals of time and weight was determined (Wf). Care was taken to ensure that there was no water present during weighing. The process was continued until saturation of final weight is observed. Swelling percentage was calculated from [8]:

$$Swelling\ percentage = \left[\frac{(W_f - W_i)}{W_i}\right] \times 100\ \% \qquad \text{.....(1)}$$

3.2.8 Water vapour transmission rate (WVTR)

Plastic bottles were taken and added with 15 ml water (Figure 1). The samples were cut in a circular shape with a diameter of 3 mm greater than the diameter of the bottle. The sample thus cut is used as a cap on the mouth of the plastic bottle. The samples were sealed to bottle using a suitable adhesive agent. The setup is weighed (Wi) and kept at 37^{o}C in an incubator for 24 hours. After 24 hours the system is weighed (Wf). WVTR was calculated as [8]:

$$WVTR = 10^6 \times \left[\frac{(W_f - W_i)}{24 \times A}\right] g/m^2/h \qquad \text{.....(2)}$$

where A is surface area of circular sample used.

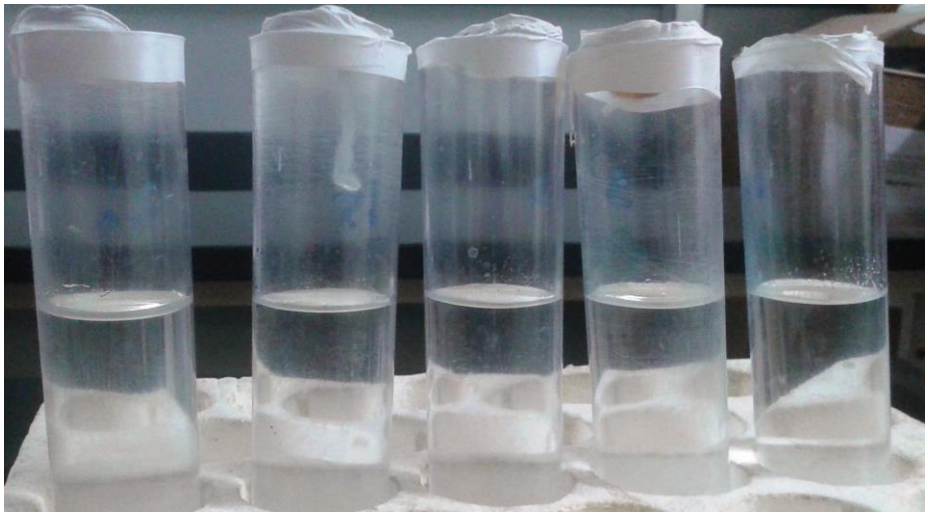

Figure 1 Experimental setup for WVTR

3.2.9 Antibacterial studies

3.2.9.1 Isolation of *Staphylococcus aureus* by using Mannitol salt agar

Mannitol salt agar medium was prepared and plated into sterile petriplates. Skin swabs were taken and serially diluted using sterile distilled water. The serially diluted samples were quadrant streaked on the prepared plates. The inoculated plates were incubated at 37^{0}C for 24 hours and observed for isolated colonies with a colouration change from red to yellow in response to acetic acid production. Nutrient broth 100 ml in

volume was prepared and autoclaved at 15 lb pressure 121^0C for 15 minutes. The broth was inoculated with 100 µl of previously maintained *E. coli* culture and *S. aureus*. The inoculated broth was kept in incubator shaker at 37^oC for 24 hours and subsequently used as inoculate in antibacterial assay.

Table 2 Mannitol salt agar composition

Ingredients	Quantity (g/l)
Peptone	10
Beef extract	1
Sodium Chloride	75
D Mannitol	10
Phenol red	0.0025
Final pH	7.4±0.2

3.2.9.2 Antibacterial assay

Petriplates with nutrient agar medium were prepared. Next the petriplates were inoculated with 100 µl overnight cultures and spread plate method was used to spread the culture. The petriplates were punched with wells using 1 ml volume tips. PVA bentonite samples were cut with diameter of 0.9 cm and the petriplates were incubated in incubator at 37^0C.

CHAPTER 4 RESULTS AND DISCUSSION

4.1 Scanning electron microscopy

The surface morphology of the PVA bentonite composites was done by scanning electron microscopy. The SEM micrographs for PVA bentonite composites with and without honey have been shown in Figure 2 and 3. The surface morphology of the composites depends upon the matrix and reinforcement concentrations and their interactions. Proper dispersion of the reinforcement in the matrix is important as it determines the various properties of the composite. Micrographs of pure PVA have showed least amount of surface roughness which may be due to the freeze-thaw method where in hydrogels are formed due to localised ice and polymeric network formations during freeze cycle and subsequent pore formations during thaw cycle. With increasing additions of OBENT into PVA matrix it is observed that the surface roughness of the samples increases. The surface roughness of the samples with increasing OBENT concentrations is due to the reason that incorporation of clay created additional crosslinking between the polymeric networks which encapsulate water within their structures to form composite hydrogels. Comparing the various PVA bentonite composite hydrogels it was found that PB20 had highest amount of surface roughness. PH sample which is formed by addition of honey to PVA leads to irregular shaped crusts and troughs on the surface of the samples. Addition of honey to PVA hydrogels has been found to increase the surface roughness to a greater extent than the PVA bentonite composites without honey due to interaction between PVA and honey that left OBENT to express surface roughness property. The undulations have been found to increase in number and frequency with increase in additions of OBENT in PVA-OBENT composites with honey. It was also observed that with increasing OBENT concentration the size of the irregularities are increasing. Highest amount of surface roughness was observed in case of PHB20 samples. A similar sort of trend was observed in case of sodium alginate and polyvinyl alcohol crosslinked hydrogels synthesized by freeze thaw method [28]. The PVA bentonite composites with honey have been found to have greater amount of surface roughness compared to PVA bentonite composites.

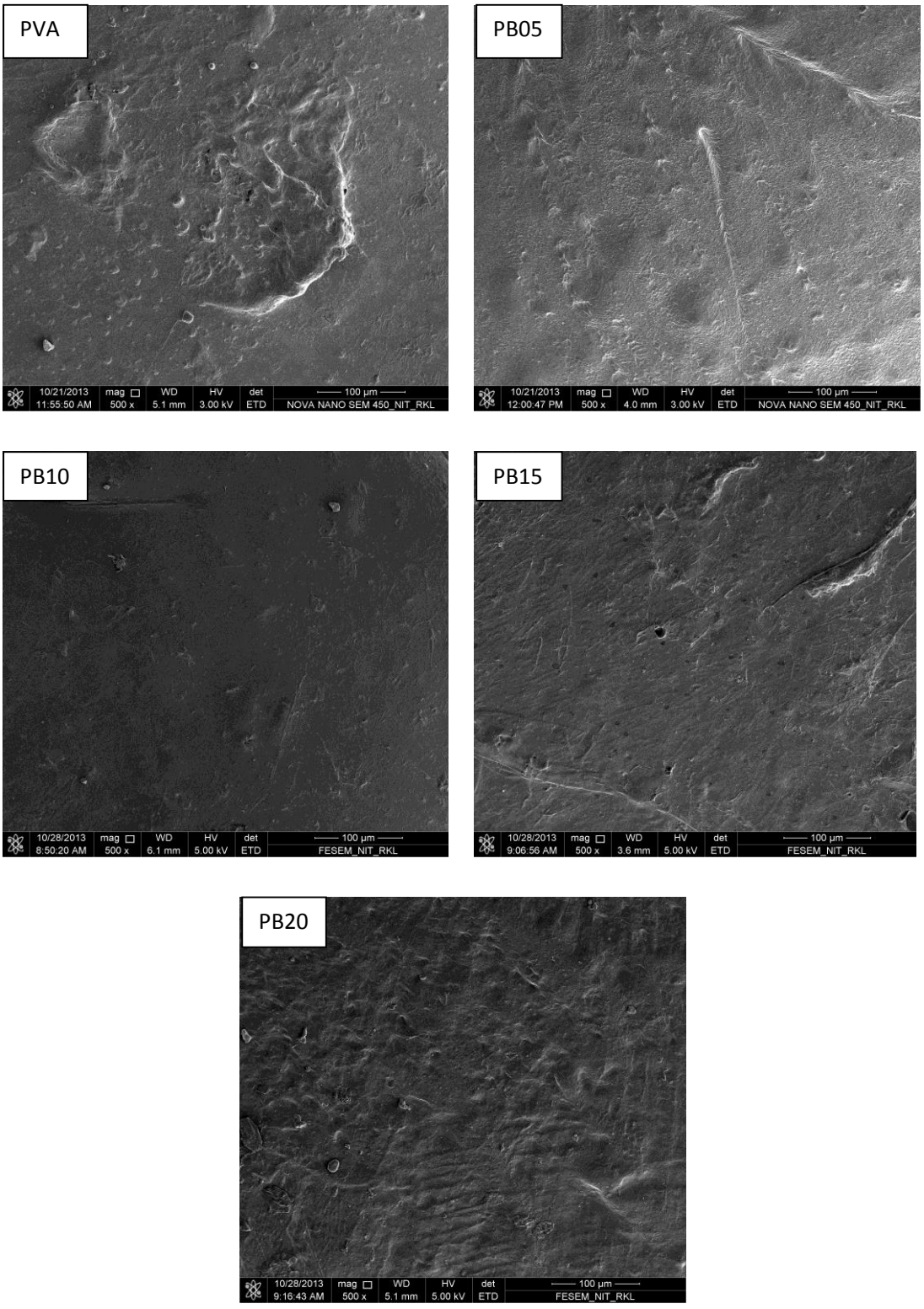

Figure 2 Scanning electron micrographs of PVA bentonite composites without honey

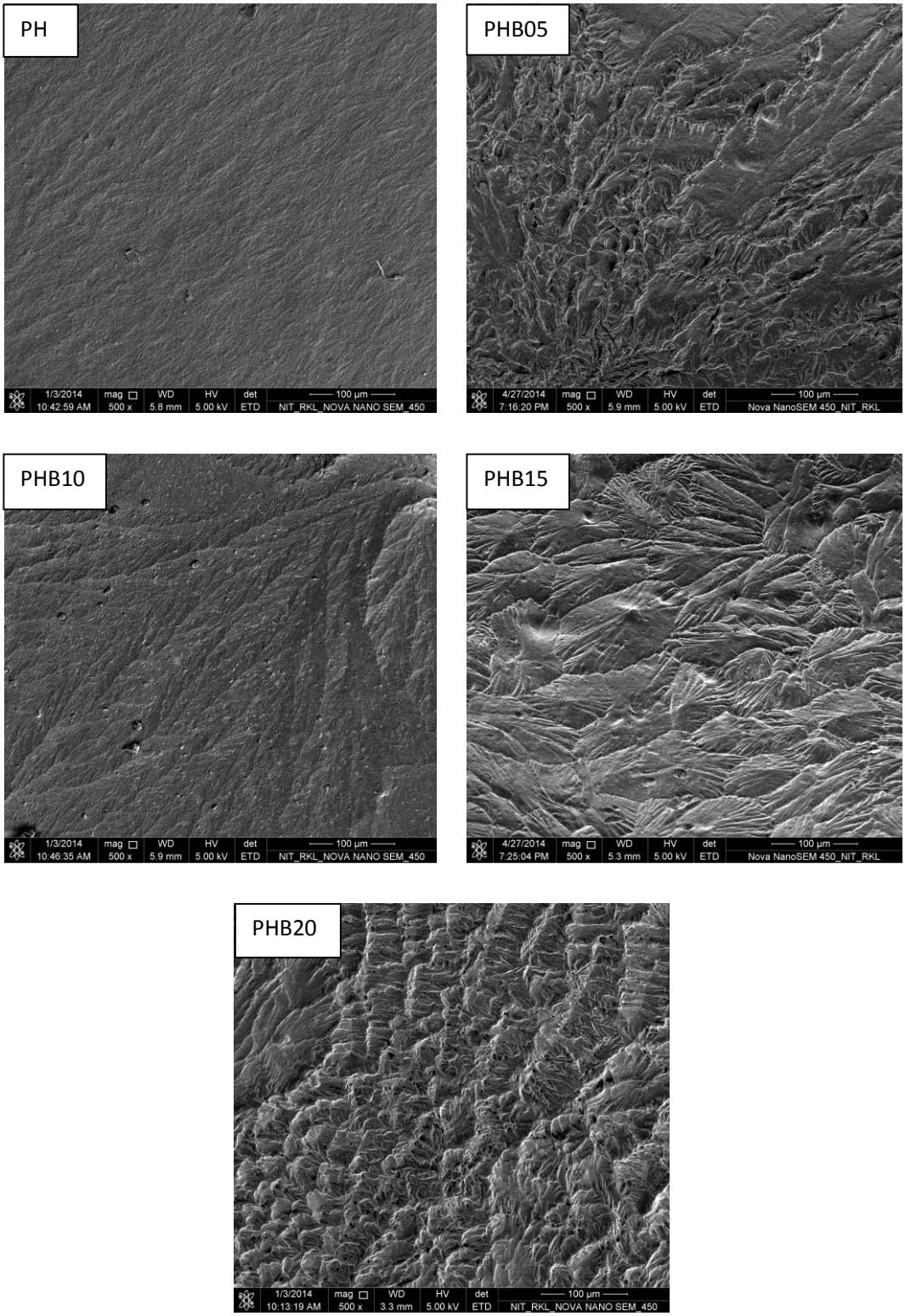

Figure 3 Scanning electron micrographs of PVA bentonite composites with honey

4.2 X-ray diffraction studies

XRD patterns were recorded to investigate any new phase formed or any contamination occurred during synthesis. The XRD patterns of PVA hydrogel, bentonite, organically modified bentonite (OBENT), PVA- organically modified bentonite (OBENT) composites and PVA organically modified bentonite (OBENT) composite with honey were obtained.

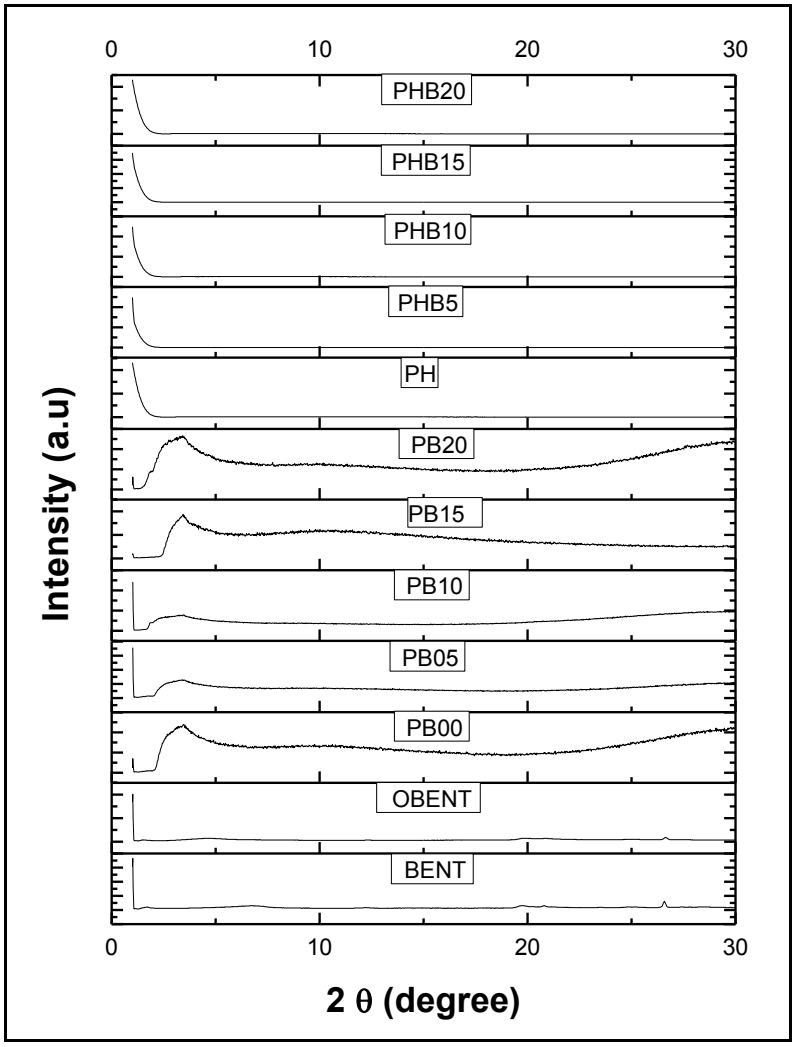

Figure 4 XRD pattern of the BENT, OBENT, PVA-OBENT composites and PVA-OBENT composites with honey

As seen in the Figure 4, bentonite has characteristic diffraction peak at 6.9° while in modified bentonite this peak was found at 4.7°. This decrease in 2θ values for OBENT in comparison to bentonite indicates the increase in d-spacing of bentonite with organic modification ($n\lambda = 2d \sin\theta$). Other peaks at 19° and 20° were also observed in both of these

samples correspond to impurities. With addition of OBENT into PVA, it was found that PVA intercalated into bentonite galleries which is evident with increased d-spacing. This increase in d-spacing was found in all other composite samples with increase in OBENT amounts. The peak of OBENT at 4.7° was found to shift towards lower 2θ values in PVA-OBENT composites. In sample PB05 a broad peak was observed at 3°. Similar peak was also found in PB10. From XRD patterns it was observed that intensity of peaks increased with increase in OBENT in PB15 and PB20 samples. So it can be said that PB05 and PB10 samples have better dispersion of OBENT in polymer matrix indicating exfoliation of OBENT within PVA matrix. With further increase of OBENT in composite samples PB15 and PB20, improper dispersion in comparison to PB05 and PB10 can be clearly seen. The extent of dispersion in PB15 and PB20 indicated the intercalation of OBENT clay. This intercalation of OBENT led to clearly visible peak around 3°. On the other hand XRD pattern of composite samples with honey showed no peaks. It might be because of reduction in crystallinity during freeze thawing method due to addition of honey. No other peaks were observed in the XRD pattern.

4.3 Fourier transform infrared spectroscopy

The FTIR spectrum is a common technique that is used to find the functional groups present in a material. The FTIR spectrum for PVA-OBENT composites hydrogels with and without honey was as shown in Figure 5. The FTIR spectrum of the samples showed the characteristic bands of both PVA and Bentonite. The band around 3500 cm^{-1} corresponds to the OH stretching vibrations of PVA. Bands at around 1098 cm^{-1} and 1734 cm^{-1} were found in PVA which correspond to C–O stretching and C=O groups respectively. The C=O groups found PVA were due to acetate groups. The band around 1000 cm^{-1} was due to -CH stretching of alkyl groups from PVA. FTIR spectrum of PVA samples blended with bentonite showed additional bands in comparison with PVA. As per the previous reports, the characteristic bands of bentonite were found around 3426 cm^{-1} which were assigned to O–H stretch and the other deformation peak of H–O–H at 1642 cm^{-1}. Sharp peaks due to Si–O stretching and vibrations were found at 1036, 521 and 467 cm^{-1}. With increased additions of OBENT in the PVA hydrogels, the intensity of the OH stretching band around 3600-3000 cm^{-1} decreased. The decreased trends might be due to the active participation of the OH groups in hydrogen bond mediated chemical interaction of PVA and OBENT [29]. The other additional peaks at 1900, 1715, 1439, 1400, 1387

and 1254 cm^{-1} were due to the chemical interactions between PVA and OBENT. Another change was observed in infrared spectra band corresponding to deformation of H–O–H at 1600 cm^{-1} which becomes narrow on increasing OBENT additions into PVA matrix. This signifies the crosslinking of PVA networks by OBENT which may lead to decrease in water absorption with increase in OBENT additions. A decrease in intensity of –OH groups in the FTIR spectra for PVA-OBENT composites can be seen with increased additions of OBENT which was due to hydrogen bonding between PVA and OBENT. PVA-OBENT composites with honey showed an increase in –OH group intensities. This increase in intensity of –OH group may due to the addition of high concentrations of sugar groups.

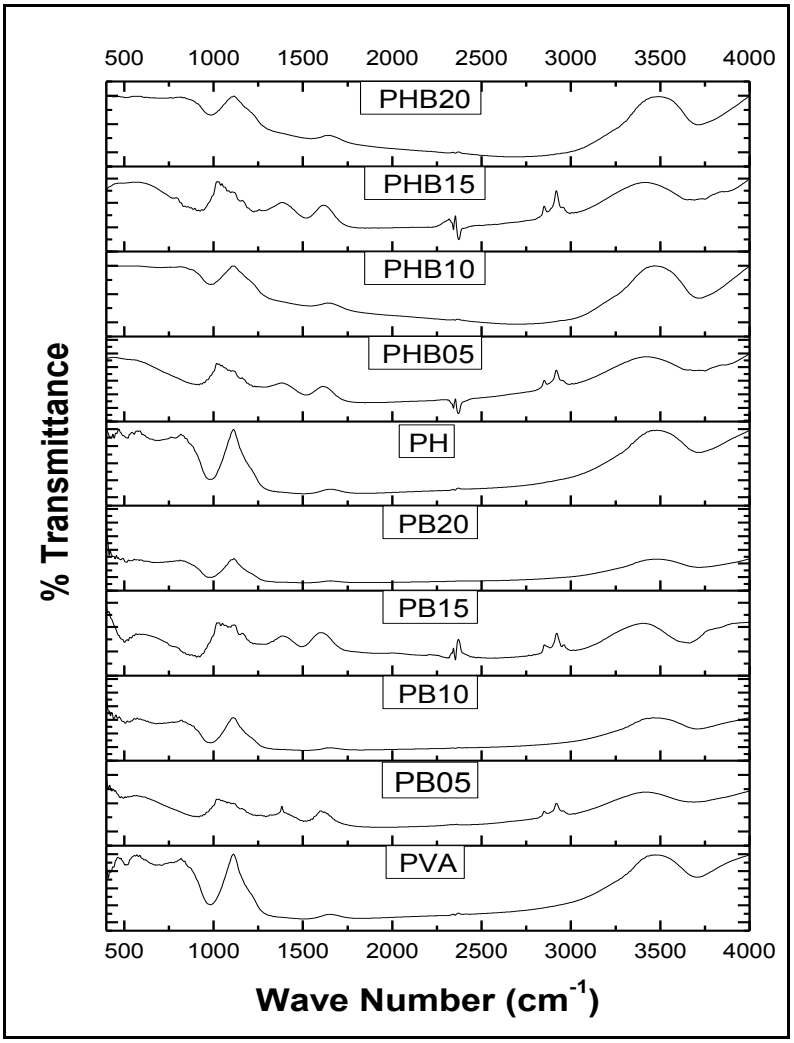

Figure 5 FTIR spectra of PVA, PVA-OBENT composites with and without honey

4.4 Swelling studies

Wound dressings must have a capacity to absorb wound fluids and exudates. It becomes necessary to characterize the swelling characteristics of hydrogels in order to decide upon its application. Hydrogels are used on a range of wounds from highly exudating wounds to dry wounds depending upon their swelling behaviour. Swelling studies also determine the period of replacement of the wound dressing. Considering the importance of swelling behaviour of composite hydrogels swelling percentages of different composite samples have been calculated. Water was used as swelling medium. Sample weights were observed at intervals of 2 hours for 34 hours at 37^0C. The influence of increasing OBENT concentrations on the swelling behaviour of PVA-OBENT composites and composites with honey was evaluated. Addition of honey into PVA-OBENT composites brings about a significant change in swelling behaviour of the matrix. Swelling percentage curves of different composites are shown in Figure 6. In case of PVA-OBENT composites it can be seen there was a steep increase in swelling percentage during the first two hours of the study which further increased gradually and saturated. In general a decrease in swelling percentage was observed with increasing OBENT amounts. PVA without OBENT showed highest swelling extent which was found to decrease as we move from PB05 to PB20. The decrease in swelling with addition of OBENT might be due to the enhanced crosslink density. These observations with respect to swelling characteristics of PVA-OBENT composite hydrogels have been found to be similar to results obtained by Kokabi et.al in their studies on PVA clay Nanocomposite hydrogels for wound dressing [8].With addition of honey in PVA-OBENT composites swelling percentage was found to be decreased as compared to composites without honey. Results have indicated that swelling percentage curves of PVA-OBENT with honey increase quickly with the immersion time. But almost after 2 hours, curves for all the samples got saturated. This can be attributed to the reason that there was a gradual release of honey into the swelling medium. The hydroxyl groups of OBENT have interaction with honey. The presence of interaction between honey and OBENT has been supported by FTIR studies. As the concentration of OBENT was increased there was greater interaction between OBENT and honey preventing the release of honey into medium. Thus PHB20 composite showed the higher swelling percentage compared to other PVA-OBENT composites with honey.

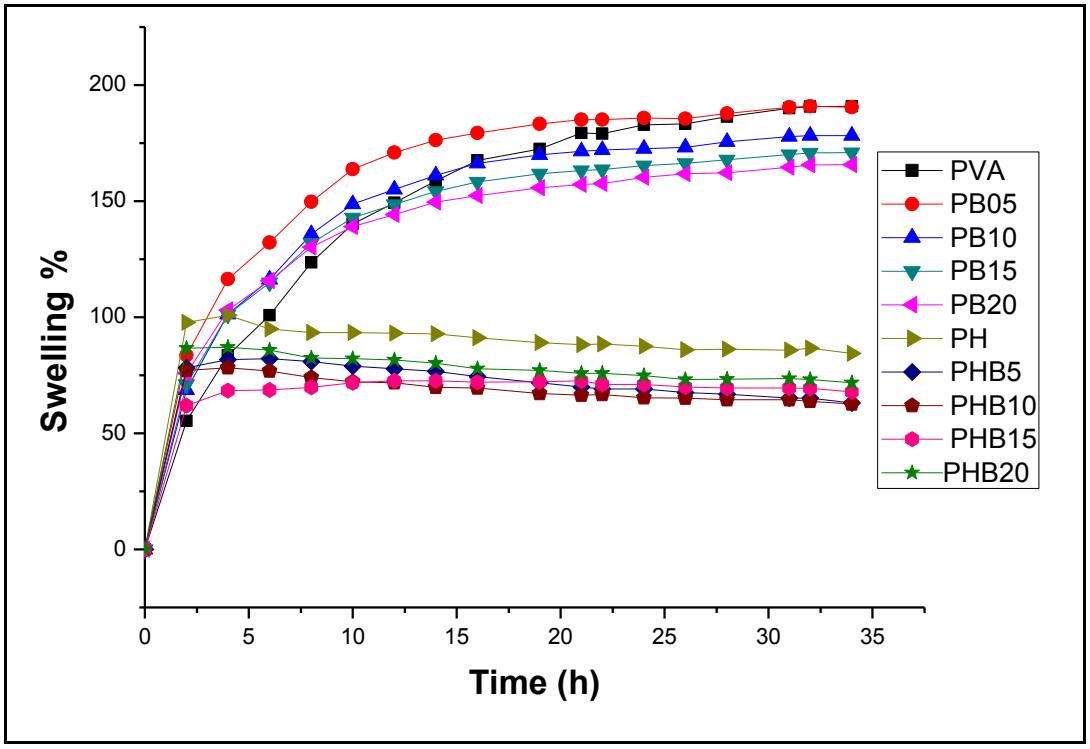

Figure 6 Swelling percentage curves of PVA, PVA-OBENT composites with and without honey

4.5 Water Vapour Transmission Rate

Water vapour transmission rate characteristics of a wound dressing determine the ability of the material to absorb and transmit the wound exudates and fluids to the external environment. Ideally a wound dressing must have WVTR values which neither allows for lowering of wound moisture nor allows for excessive water retention are preferred. A low WVTR wound dressing allows for greater amount wound fluids to be in the environment which increases the chances of secondary infections. The process of transport of the exudates determines the amount of moisture provided by the dressing to the wound. Maintenance of a moist environment is one of the ideal wound dressing characteristics. Importance of moist environment for wound healing was practically explored by Dr. Winter in 1962. The maintenance of moist environment by wound dressings leads to cell migration and accelerated rate of wound healing. The material of the wound dressing plays a major part in the water vapour transmission rate values.

Table 3 Water vapour transmission values obtained for various PVA Bentonite composites with or without honey

SL No.	Sample Code	WVTR values in $g/m^2/h$
1	PVA	7.072
2	PB05	7.8
3	PB10	9.140
4	PB15	9.310
5	PB20	9.430
6	PH	4.71
7	PHB5	3.412
8	PHB10	3.394
9	PHB15	3.390
10	PHB20	3.394

The water vapour transmission rate values for normal skin and diseased skin have been reported to be 8.5 $g/m^2/h$ to 11.6 $g/m^2/h$ [30]. The WVTR values obtained for various PVA-OBENT composites with and without honey are shown in Table 3. The WVTR values obtained for PB05, PB10, PB15 and PB20 were found to be near the range of WVTR values. The Water vapour transmission rate values of PVA bentonite composites with honey have been found to be towards the lower side of the range. The PVA bentonite composites with honey could be used for low exudate and fluid releasing dry wounds. The PVA bentonite composites without honey could be in cases of high exudate wounds [8].

4.6 Antibacterial studies

Our body has a large diversity of normal microbial flora and fauna which help in normal functioning and maintenance. Under diseased conditions these organisms take advantage of the situation to cause infection and are called as opportunistic pathogens. Under conditions where skin has been damaged and wounds prevail, secondary infections by microbes are a common phenomenon. In cases where in the wounds are not protected from secondary infections the time for wound healing increases. So, it becomes important

to access the property of wound dressings to prevent wound infections. In this study nutrient agar diffusion assay was used to evaluate the property of PVA-OBENT composites hydrogels with or without honey against gram negative *E. coli* and gram positive *S. aureus* [30]. Most of the common skin infections involve *S. aureus*, which is a gram positive bacterium. The common gram negative bacteria *E. coli* is used as standard organism for antimicrobial activities. Hence these two organisms were selected as models for antibacterial properties. PVA-OBENT composites were tested against *S. aureus* and it was found that PB15 and PB20 with 15% and 20% OBENT additions had highest zones of inhibitions as shown in Figure 7. PVA sample showed the least zone of inhibition. Similar sort of trend (as shown in Figure 8) was observed in PVA-OBENT composites with honey. Highest zone of inhibition was shown by PHB20 samples whereas least zone of inhibition was shown by PH samples. PVA-OBENT composite hydrogels with and without honey were tested for antibacterial properties against *E. coli* as shown in Figure 9 and 10 respectively. PVA-OBENT composites have been found to be effective against *E. coli* as PB20 showed highest zone of inhibition and PVA had the lowest values. Similar trends were observed when PVA-OBENT composites with honey where it was found that PHB20 showed highest zone of inhibition and PH samples showed the least zones of inhibition. Thus it can be said that with increase in OBENT concentrations in PVA-OBENT composites with and without honey increases with increasing additions of OBENT against *E. coli* as well as in *S. aureus.* Earlier reports have stated that clay minerals in their nanomaterial forms have been effective as antibacterial properties.

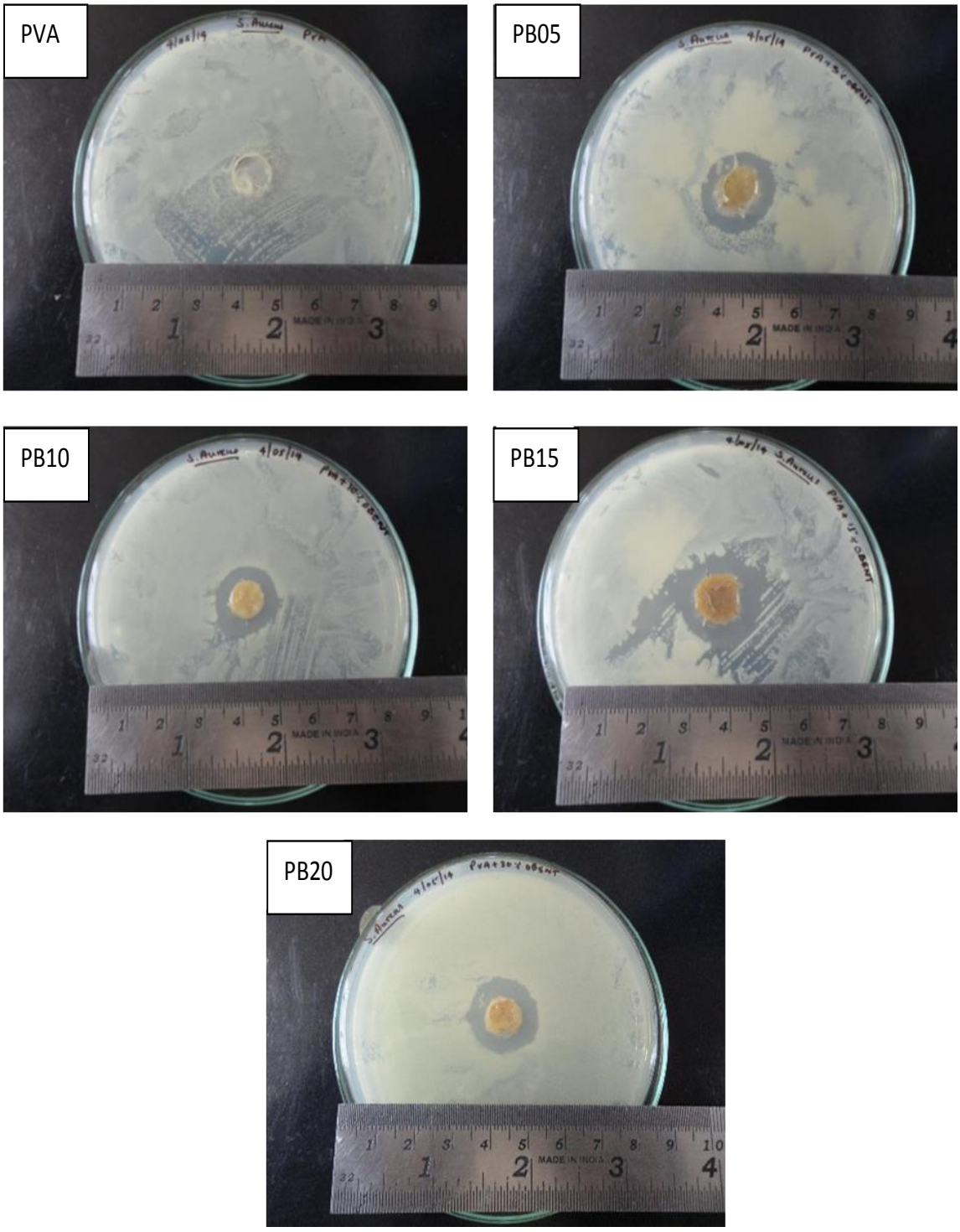

Figure 7 Results showing antibacterial activity of PVA-OBENT composites against *S. aureus*

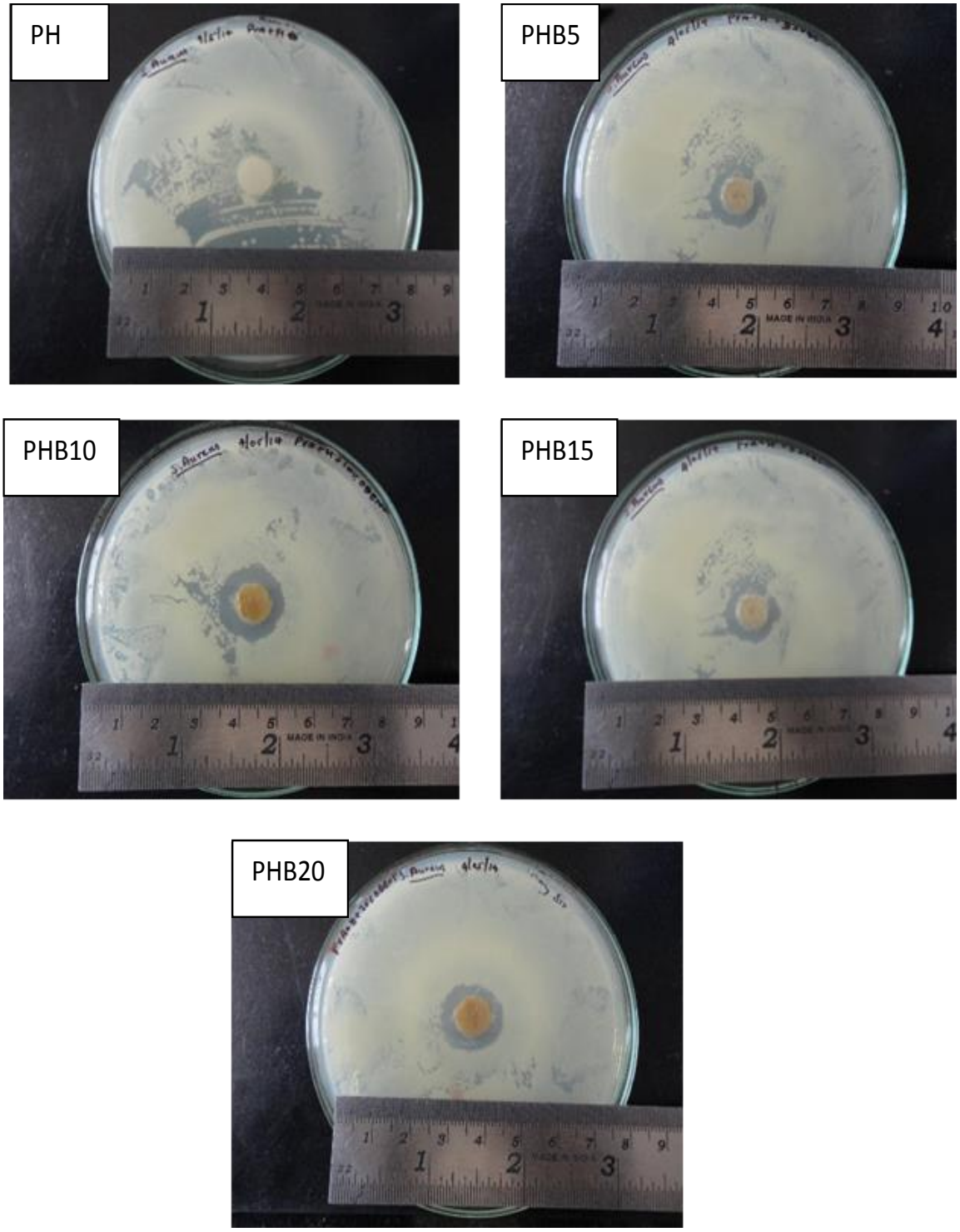

Figure 8 Results showing antibacterial activity of PVA-OBENT composites with honey against *S. aureus*

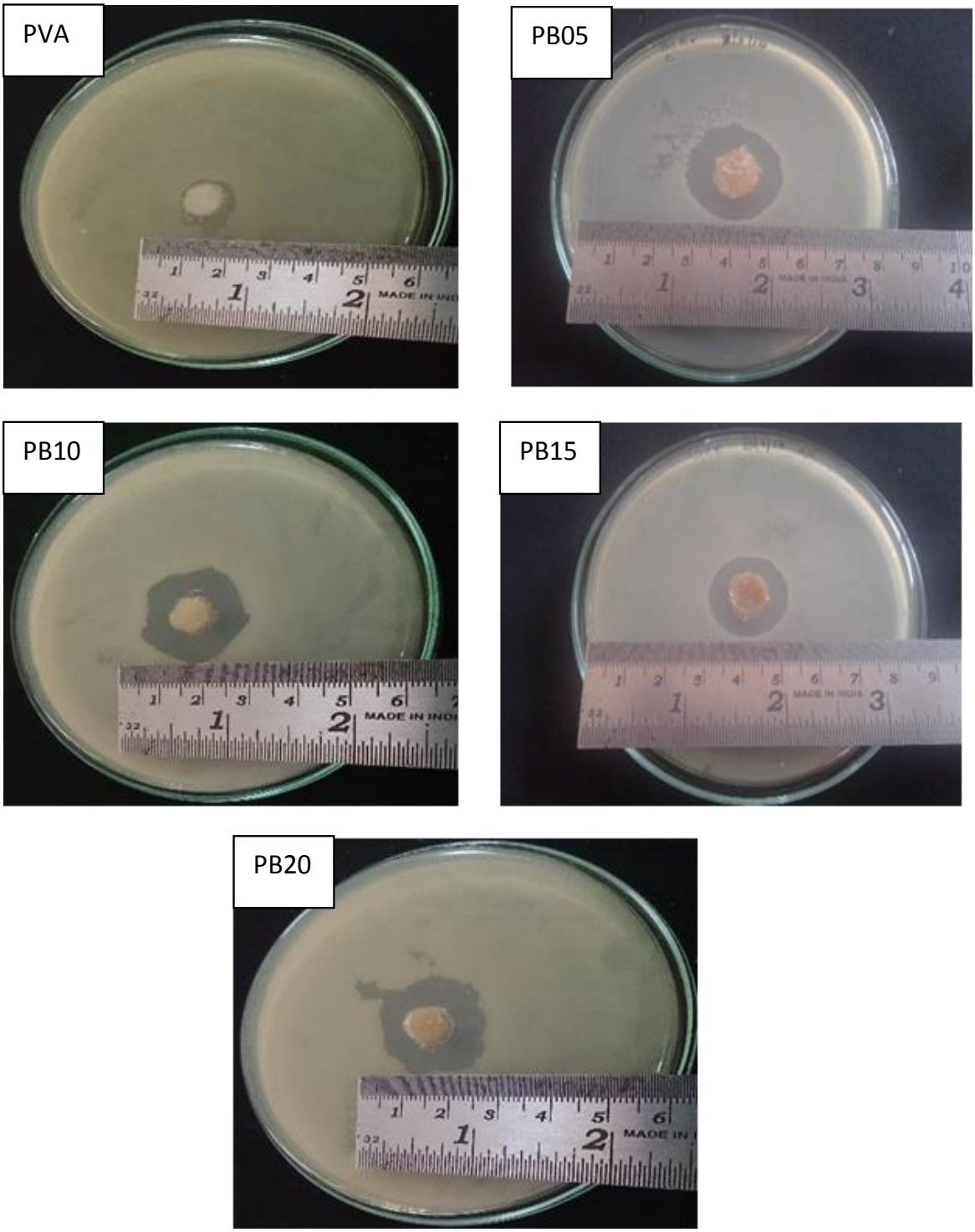

Figure 9 Results showing antibacterial activity of PVA-OBENT composites against *E.coli*

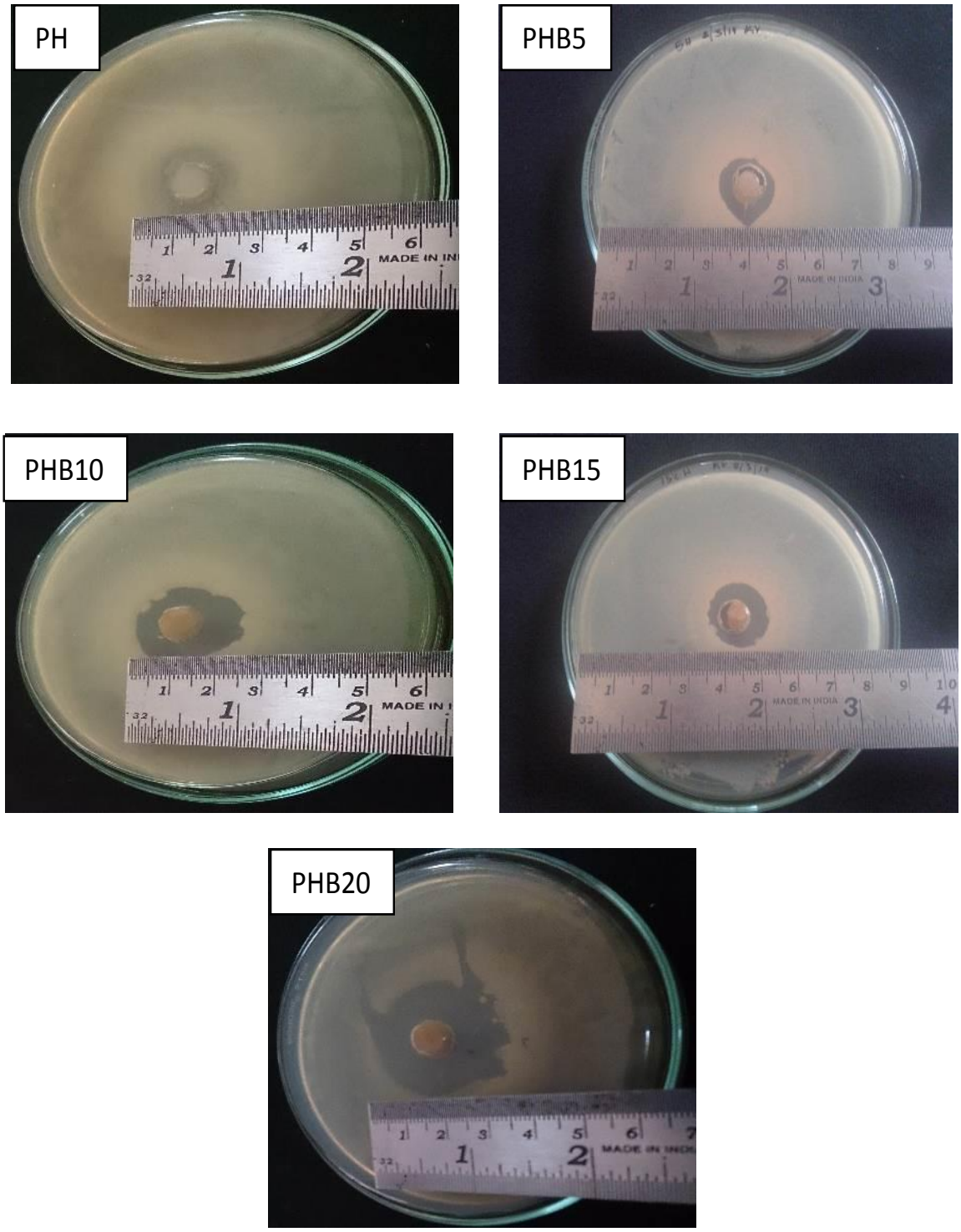

Figure 10 Results showing antibacterial activity of PVA-OBENT composites with honey against *E. coli*

In past clays materials have been used in treatment of skin infections and ulcer treatment [31]. It has to be noted that bentonite clay was modified by using CTAB and CTAB has been reported to to have antibacterial properties. So, we can converge at a point that

OBENT functionalised with CTAB increased the antibacterial properties of PVA hydrogels. In order to increase the wound dressings property to enhance protection against secondary infections antibiotics were incorporated. Honey has been found to have antibacterial properties against antibiotic resistant bacteria. Hence the honey was used as an additional agent to increase the antibacterial property of wound dressing material. It is evident that minimum of 40% of honey has shown maximal antibacterial effects [19].

SUMMARY AND CONCLUSION

PVA Bentonite composite hydrogels with and without honey have been prepared by freeze thaw method using different concentrations of organically modified bentonite (OBENT). Polyvinyl alcohol (PVA) at 15% (w/v) has been ideally used for hydrogel formation. The synthesized PVA bentonite hydrogels have been characterized using scanning electron microscopy, X-ray powder diffraction and fourier transform infrared spectroscopy. The wound dressing characteristics were also evaluated by performing swelling studies, water vapour transmission rate studies and antibacterial studies. Scanning electron microscopy results show an increased surface roughness with increase in organically modified bentonite additions in PVA bentonite composite hydrogels with and without honey samples. The X-ray powder diffraction studies of the PVA bentonite composite hydrogels show the left side peak shift and increase in d spacing due to intercalation of polymer within the OBENT interlayer spaces of the various PVA bentonite composites. Fourier transform infrared spectroscopy analysis of the PVA bentonite composites signifies the interactions between PVA, honey and organically modified bentonite. The swelling studies and water vapour transmission studies show that the PVA bentonite composites hydrogels without honey could be used for high exudate and fluid releasing wounds whereas PVA bentonite composite hydrogels with honey could be used for dry or less exudate wounds. PVA bentonite composite hydrogels with honey and without honey show good antibacterial behaviour with respect to *E. coli* and *S. aureus* bacteria. The synthesized composite hydrogel was found to satisfy various ideal wound dressing characteristics like moisture maintenance, water absorption and protection from secondary infections proving their wound dressing potential.

REFERENCES

1. Heather L. Orsted and D.K., Louise Forest Lalande, Marie Françoise Mégie, *Basic Principles of Wound Healing.* Wound Care Canada, 2004. **9**(2): p. 4-12.

2. Queen, D., *Technology update: Understanding Hydrofiber® Technology.* Wounds International, 2010. **1**(5).

3. Abdelrahman, T. and H. Newton, *Wound dressings: principles and practice.* Surgery (Oxford), 2011. **29**(10): p. 491-495.

4. Hassan, C. and N. Peppas, *Structure and Applications of Poly(vinyl alcohol) Hydrogels Produced by Conventional Crosslinking or by Freezing/Thawing Methods*, in *Biopolymers · PVA Hydrogels, Anionic Polymerisation Nanocomposites*. 2000, Springer Berlin Heidelberg. p. 37-65.

5. Kirschner, C.M. and K.S. Anseth, *Hydrogels in healthcare: From static to dynamic material microenvironments.* Acta Materialia, 2013. **61**(3): p. 931-944.

6. Rosiak, J.M., Ulański, P., Pajewski, L.A., Yoshii, F. and Makuuchi, K., *Radiation formation of hydrogels for biomedical purposes. Some remarks and comments.* Radiation Physics and Chemistry, 1995. **46**(2): p. 161-168.

7. Kickhöfen, B., Wokalek, H., Scheel, D. and Ruh, H., *Chemical and physical properties of a hydrogel wound dressing.* Biomaterials, 1986. **7**(1): p. 67-72.

8. Kokabi, M., Sirousazar M., and Hassan Z.M., *PVA–clay nanocomposite hydrogels for wound dressing.* European polymer journal, 2007. **43**(3): p. 773-781.

9. Corkhill, P.H., C.J. Hamilton, and Tighe, B.J., *Synthetic hydrogels VI. Hydrogel composites as wound dressings and implant materials.* Biomaterials, 1989. **10**(1): p. 3-10.

10. Stammen, J.A., Williams, S., Ku, D.N., and Guldberg, R.E., *.Mechanical properties of a novel PVA hydrogel in shear and unconfined compression.* Biomaterials, 2001. **22**(8): p 799-806.

11. Wang, R., S. Zheng, and Zheng, Y.G., *Polymer matrix composites and technology*. 2011: Elsevier, Woodhead Publishing.

12. Maribel I. Baker, S.P.W., Zvi Schwartz and Barbara D. Boyan, *A review of polyvinyl alcohol and its uses in cartilage and orthopedic applications.* Journal of biomedical materials research b: applied biomaterials, 2012. **9999B**(00): p. 1-7.

13. E. G Crispim, J.F.P., A. R Fajardo, E. R. F Ramos, T. U Nakamura, C. V Nakamura, and E.C.M. A. F Rubira, *Hydrogels based on chemically modified poly(vinyl alcohol)(PVA-GMA) and PVA-GMA/chondroitin sulfate:Preparation and characterization.* Express Polymer Letters, 2012. **6**(5): p. 383-395.

14. Siddhi Gupta, T.J.W. and A.A. Sinha, *Evolution of PVA gels prepared without crosslinking agents as a cell adhesive surface.* J Mater Sci: Mater Med, 2011(22): p. 1763-1772.

15. Peppas, S.R.S.a.N.A., *Poly(vinyl alcohol) hydrogels prepared by freezing-thawing cyclic processing.* Polymer, 1991. **33**(18): p. 3932-3936.

16. Kiliaris, P. and C.D. Papaspyrides, *Polymer/layered silicate (clay) nanocomposites: An overview of flame retardancy.* Progress in Polymer Science, 2010. **35**(7): p. 902-958.

17. Koo, J.H., *Polymer nanocomposites*. 2006: McGraw-Hill Professional Pub. New York.

18. Paul, D.R. and L.M. Robeson, *Polymer nanotechnology: Nanocomposites.* POLYMER, 2008. **49**(15): p. 3187-3204.

19. Kwakman, P.H.S. and S.A.J. Zaat, *Antibacterial components of honey.* IUBMB Life, 2012. **64**(1): p. 48-55.

20. Marks J Fau - Harding, K.G., L.E. Harding Kg Fau - Hughes, and L.E. Hughes, *Staphylococcal infection of open granulating wounds.* The British Journal of Surgery, 1987. **74**(2): p. 95-97.

21. Moody, A., *Use of a hydrogel dressing for management of a painful leg ulcer.* Br J Community Nurs, 2006. **11**(6): p. S12, S14, S16-17.

22. Kudo, S., E. Otsuka, and A. Suzuki, *Swelling behavior of chemically crosslinked PVA gels in mixed solvents.* Journal of Polymer Science Part B: Polymer Physics, 2010. **48**(18): p. 1978-1986.

23. Singh, B. and L. Pal, *Radiation crosslinking polymerization of sterculia polysaccharide–PVA–PVP for making hydrogel wound dressings.* International Journal of Biological Macromolecules, 2011. **48**(3): p. 501-510.

24. Varshney, L., *Role of natural polysaccharides in radiation formation of PVA–hydrogel wound dressing.* Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms, 2007. **255**(2): p. 343-349.

25. Sapalidis, A.A., Katsaros, F.K., Steriotis, Th.A. and Kanellopoulos, N.K., *Properties of poly(vinyl alcohol)—Bentonite clay nanocomposite films in relation to polymer–clay interactions.* Journal of Applied Polymer Science, 2012. **123**(3): p. 1812-1821.

26. Pique, T.M., *Water soluble nanocomposite films based on poly(vinyl alcohol) and chemically modified montmorillonites.* Journal of Composite Materials, 2014. **48**(5): p. 545-553.

27. Molan, P.C., *Potential of honey in the treatment of wounds and burns.* American Journal of Clinical Dermatology, 2001. **2**(1): p. 13-19.

28. Kamoun, E.A., Kenawy, El-Refaie S. and Tamer, T.M., *Poly (vinyl alcohol)-alginate physically crosslinked hydrogel membranes for wound dressing applications: Characterization and bio-evaluation.* Arabian Journal of Chemistry, 2013.

29. İşçi, S. and Unlu, C.H., *Rheology and structure of aqueous bentonite-polyvinyl alcohol dispersions.* Bulletin of Materials Science, 2006. **29**(5): p. 449-456.

30. Gonzalez, J.S. and Maiolo, A.S., *Composite Gels Based on Poly (Vinyl alcohol) for Biomedical Uses.* Procedia Materials Science, 2012. **1**(0): p. 483-490.

31. Carretero, M.I., *Clay minerals and their beneficial effects upon human health. A review.* Applied Clay Science, 2002. **21**(3–4): p. 155-163.